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Automatic Surface Inoculation of Agar Trays[1]

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A machine is described which automatically inoculates a plastic tray containing agar media with a culture by use of either a conventional inoculating loop or a cotton swab. Isolated colonies were obtained with an inoculating loop when a heavy inoculum (10^9 cells/ml) was used or with a cotton swab when a light inoculum (ca. 10^4 cells/ml) was used. Trays containing combinations of differential or selective media were used to (i) separate mixtures of gram-positive and gram-negative bacteria, (ii) facilitate isolation of organisms from clinical specimens, and (iii) compare colony growth characteristics of pure cultures. The design of the machine is simple, it is easy to use, and it relieves the operator from the manual task of streaking cultures.

One of the most frequently performed operations in a microbiology laboratory is the spreading of an inoculum over a culture plate to obtain isolated colonies. Although it would be desirable to mechanize this procedure, only a limited number of techniques have been developed, and these were based on spreading the inoculum by moving a pipette or bacteriological loop along the radius of a rotating agar plate (2; H. R. Attebery and W. T. Carter, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 11, 1972). Trotman (3) described an automatic device based on this principle which relieved the operator from the manual task of streaking the plate, but his technique still has the disadvantages that the inoculum must be added separately to the plate, additional plates have to be streaked if more than one medium is used, and, finally, the instrument described is relatively complex. Recently, Campbell (1) described a new system for plating bacteria in which the sample was applied in a decreasing concentration in the form of an Archimedes spiral. This procedure restricts all bacteria to the path of the spiral, with the highest concentration per unit length at the center of the plate and the lowest concentration at the edge.

In this communication, we describe an instrument for the automatic inoculation of agar trays which is simple in design and easy to operate, and which allows the technician to carry on with other activities while the trays are being inoculated. It employs either a conventional inoculating loop or a cotton swab for uniform inoculation of agar media and colony isolation. With the technique described, combinations of media can be employed in one tray to isolate or differentiate between bacterial types. Finally, this technique permits visualization of the overall qualitative and, to some extent, quantitative relationships of the various bacterial types in the sample tested.

[1] Patent application has been approved for filing by the National Aeronautics and Space Administration.

MATERIALS AND METHODS

Description and operation of the agar-inoculating machine. Basically, the apparatus shown in Fig. 1 consists of a motor-driven carriage that moves a plastic tray containing agar media past an inoculating loop or a cotton swab which inoculates the agar surface. Its overall dimensions are 4 inches (ca. 10.1 cm) wide, 21 inches (ca. 53.3 cm) long, and 10 inches (ca. 25.4 cm) high. The carriage is mounted on a double-railed track and is attached by a cable to the carriage drive motor (model 43A106-1, Globe Industries, Dayton, Ohio) assembly. The carriage speed is controlled by adjusting a rheostat, and the times required for total length of carriage travel of 9 inches (ca. 22.8 cm) are as follows: low speed, 105 sec; medium speed, 80 sec; and high speed, 65 sec. The swabbing motor (model 6907, Globe Industries) is mounted in a single gimbal. Attached to the swabbing motor is a swabbing crank with a hole and set screw for holding the inoculating loop or cotton swab. Pressure and angle of the loop or cotton swab relative to the agar surface can be altered by moving the swabbing motor back and forth or up and down. An actuator motor (modified part DYLM 4331-20, Barber Coleman Co., Rockford, Ill.) lifts or lowers the inoculating loop or cotton swab through an arm; the other electrical components are three 14.0-v DC relays (Allied Control Co., New York, N.Y.) and one

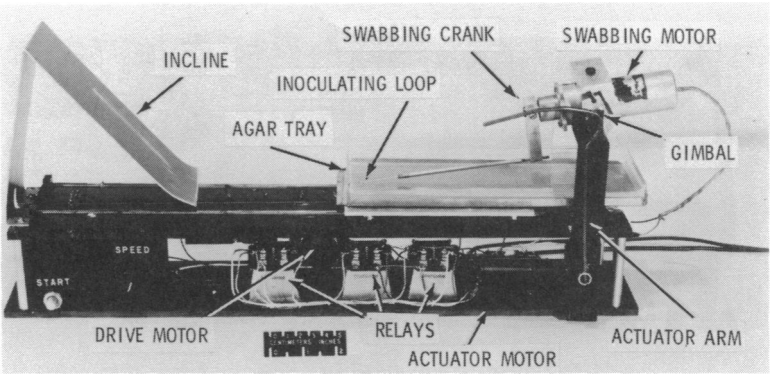

FIG. 1. *Major components of the automatic agar inoculator.*

semiconductor diode. The electrical energy to drive the system is 15 v at 2 amperes and is provided from a DC power supply (model HH32-15, Trygon Electronics, Roosevelt, N.Y.); if portability is a requirement, suitable batteries can be substituted for the power supply. The plastic trays, 3¼ inches (ca. 8.4 cm) wide and 9⅝ inches (ca. 24.4 cm) long (Read Plastics Corp., Rockville, Md.), contain dividers to separate the selective or differential media and were sterilized with either ethylene oxide or ultraviolet light.

To operate the machine, the inoculating loop or cotton swab to be processed is attached to the swabbing crank; a tray containing the appropriate media is placed in the carriage and the lid is removed. Power is applied through the start switch which activates the swabbing, drive, and actuator motors. The following sequence of events then takes place, as shown in Fig. 2. The actuator arm lowers the loop to the agar surface and, as the tray proceeds forward, the loop, which is in continuous contact with the agar surface, inoculates the media by a combined 3-inch (ca. 7.6 cm) stroke from side to side plus a spiraling action in the y-z plane about the longitudinal x axis. The tray then proceeds up the 45° incline so that the trailing edge of the tray does not interfere with the streaking action. When the tray is completely inoculated, a microswitch reverses tray direction and at the same time activates the actuator motor which permits the actuator arm to raise the loop from the agar surface. After the carriage returns to its original position, a microswitch deactivates all motors. All operations are conducted inside a clear plastic box which is 15 inches (ca. 38.1 cm) high, 12 inches (ca. 30.4 cm) wide, and 28 inches (ca. 71.1 cm) long. The box is equipped with two ultraviolet lamps (G15T8, General Electric, Richmond Heights, Ohio) which were activated between tests.

Bacterial cultures. The following organisms used in this study were obtained from the American Type Culture Collection (Rockville, Md.); *Staphylococcus aureus* 12600, *S. epidermidis* 155, *Escherichia coli* 12014, *Serratia marcescens* 13880, *Pseudomonas aeruginosa* 17934, *Klebsiella pneumoniae* 13046, and *Proteus mirabilis* 12453. All cultures were maintained at 5 C on Trypticase soy agar slants (TSA; BBL) and transferred monthly.

Bacterial counts. Counts were made from 24-hr Trypticase soy broth (TSB, BBL) cultures by spreading appropriate 10-fold dilutions on TSA. Plate incubation was at 35 C for 24 hr except in the case of *S. epidermidis*, which was incubated for 48 hr. Colonies were counted with a Quebec colony counter (American Optical Co., Buffalo, N.Y.).

Tray inoculation and incubation. Trays containing TSA or a combination of selective or differential media, i.e., blood agar, eosin methylene blue (EMB, BBL), tellurite glycine agar (TGA, Difco), etc., were inoculated with a 0.01-ml standardized inoculating loop (Scientific Products, Evanston, Ill.) or a cotton swab (SWUBE, 2021, Falcon Plastics) from which excess fluid was expressed against the side of the tube. Trays were incubated for 24 hr at 35 C except with *S. epidermidis*, which was incubated for an additional 24 hr. The trays were photographed with a multipurpose industrial view camera (Polaroid MP-3Land Camera, Polaroid Corp., Cambridge, Mass.).

RESULTS AND DISCUSSION

Inoculation patterns. The inoculation patterns for a loop and a cotton swab were distinctly different (Fig. 3). In the case of a swab, the pattern consisted of a brush-type effect, which resulted from the swab's maintaining almost continuous contact with the agar surface during the side to side streaking action. It was only during the spiraling action along the longitudinal axis that the swab tip touched the agar. This type of inoculation resulted in a uniform distribution of cells from one end of the tray to the other.

With the inoculating loop, however, the major portion of the inoculum was deposited on the agar surface during the initial streaking, with a gradual release of cells as streaking continued the length of the tray. During the initial stages of streaking, as the flat side of the loop contacted the agar surface, the film inside the loop ruptured and released the majority of cells. As

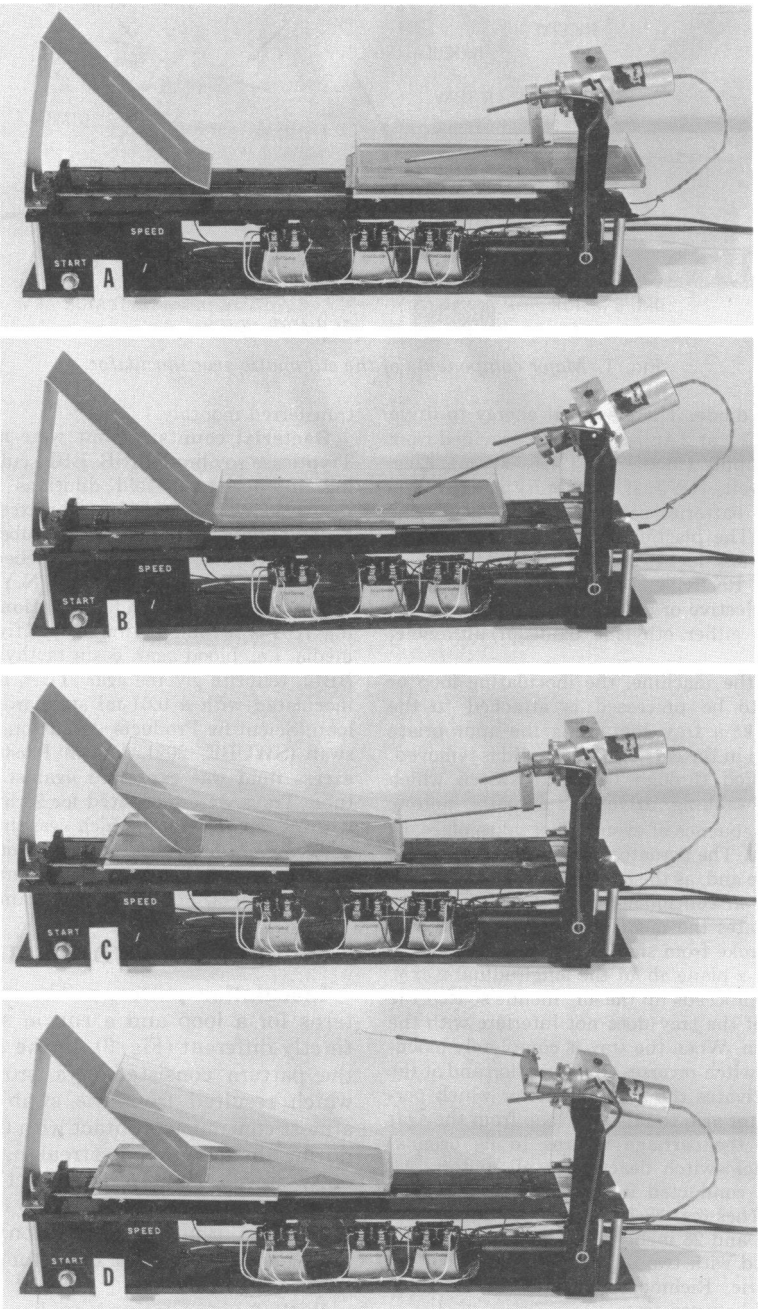

FIG. 2. *Sequence of inoculating agar trays: (A) actuator arm lowers inoculating loop to agar surface, (B) tray is entering incline, (C) inoculation is completed (note that loop is not touching edge of tray), and (D) actuator arm has raised loop and tray returns to original position.*

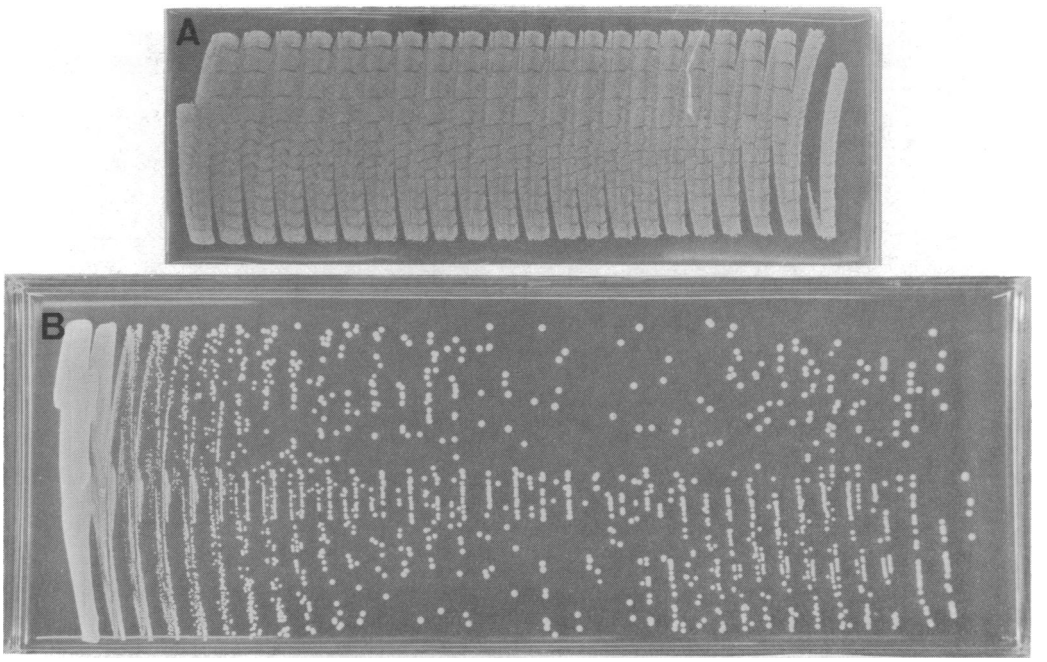

FIG. 3. *Inoculation patterns for a cotton swab (A) and inoculating loop (B). The inoculum in both cases was 10^8 cells/ml. The organisms were Staphylococcus aureus (A) and S. epidermidis (B).*

the loop proceeded back across the agar surface, the spiraling action allowed only the leading edge of the loop tip to remain in contact with the agar, and at this point single cells or small clumps were released which resulted in isolated colonies. The production of isolated colonies by the loop method was also influenced by the rate at which the tray traveled; optimal isolation occurred at a low speed of 105 sec for total length of carriage travel. On the other hand, tray speed did not appear to influence colony isolation with a cotton swab when a light inoculum was employed.

Inoculum size. The effect of inoculum size on the distribution of cells and colony isolation for the loop and swab methods is shown in Fig. 4. Our experience with a wide variety of species and numerous tests has shown that to obtain isolated colonies with a cotton swab, which retained ca. 0.15 ml of liquid, the inoculum must contain $\leq 10^4$ cells/ml; with a larger inoculum, a uniform lawn of growth resulted. We have also noted that with an optimal inoculum for isolation, ca. 10^4 cells/ml, the distribution of colonies was uniform throughout the tray as a result of the squeegee-like action of the swab against the agar surface. With the inoculating loop, however, although isolated colonies were consistently obtained with a heavy inoculum (10^8 cells/ml), the distribution was not as uniform as noted when the cotton swab was used with an inoculum of ca. 10^4 cells/ml. As shown in Fig. 4, the number of isolated colonies with a loop decreased as the inoculum size was reduced, owing to the fact that (i) most of the cells were released during initial contact of the loop with the agar and (ii) the loop volume represented only one-hundredth of the original inoculum.

In those cases in which it was necessary to obtain isolated colonies from a swab containing a heavy inoculum, i.e., 10^8 to 10^9 cells/ml, we have achieved excellent isolation of colonies by use of the following technique: the swab was rubbed over the agar surface near the top of the tray, a sterile loop was allowed to pass through the swabbed area, and the rest of the tray was streaked (Fig. 5).

Combinations of media. One of the attractive features of this instrument is the capability to incorporate various combinations of differential and selective media in one tray, as shown in Fig. 6. Although we have used trays with two and three different types of media, the number of combinations could be tailored to meet experimental or clinical requirements. For example, in dealing with a mixture of gram-positive and gram-negative bacteria, we have satisfactorily used a combination of blood agar to grow both organisms, azide blood agar or

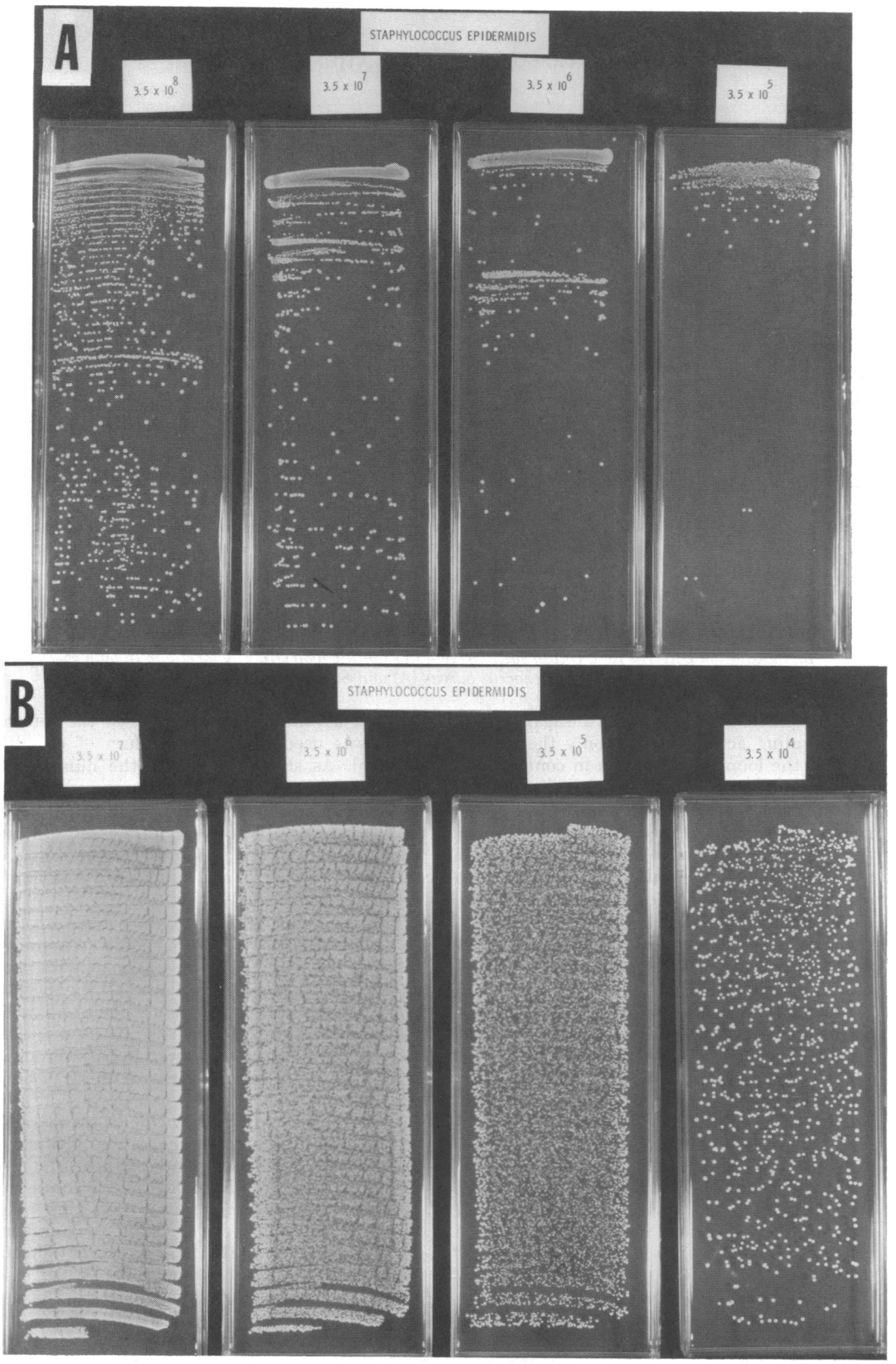

FIG. 4. *Effect of inoculum size (cells per milliliter) on cell distribution and colony isolation for (A) an inoculating loop and (B) a cotton swab. Note the inverse relationship between inoculum size and colony isolation for the loop and cotton swab.*

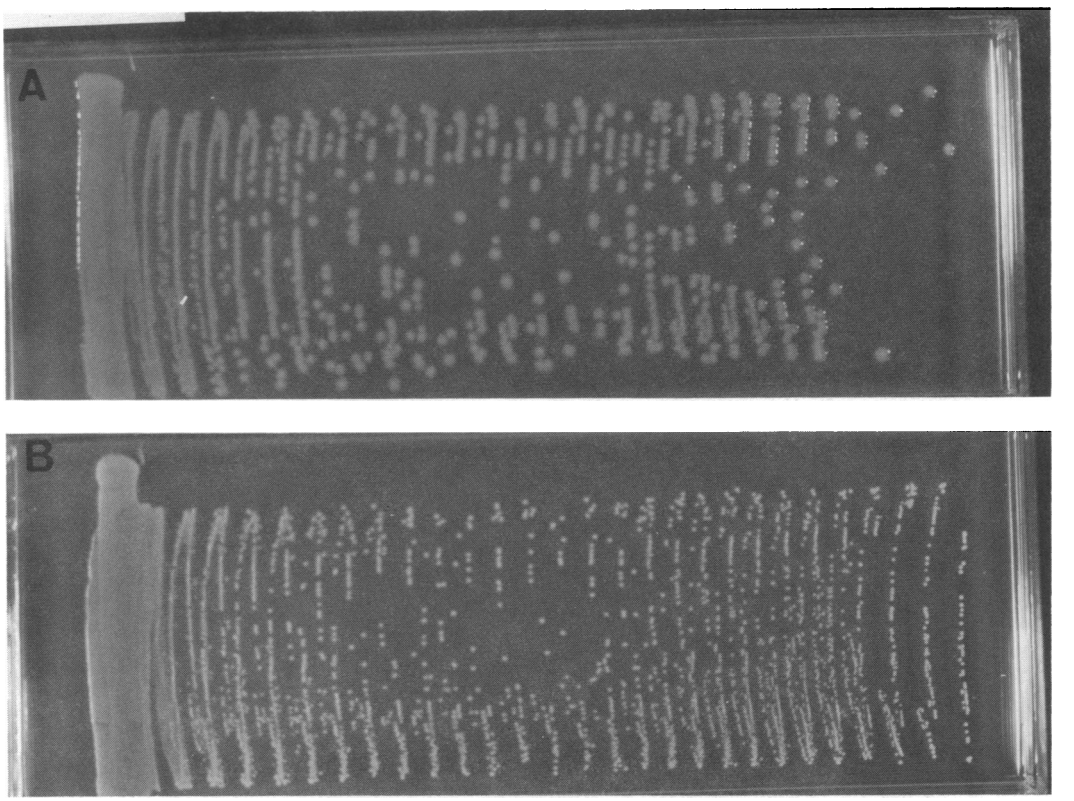

FIG. 5. *Colony isolation resulting from rubbing a cotton swab across the agar surface followed by streaking with a sterile inoculating loop. (A) E. coli; (B) S. aureus. The swab inoculum was 10^8 to 10^9 cells/ml.*

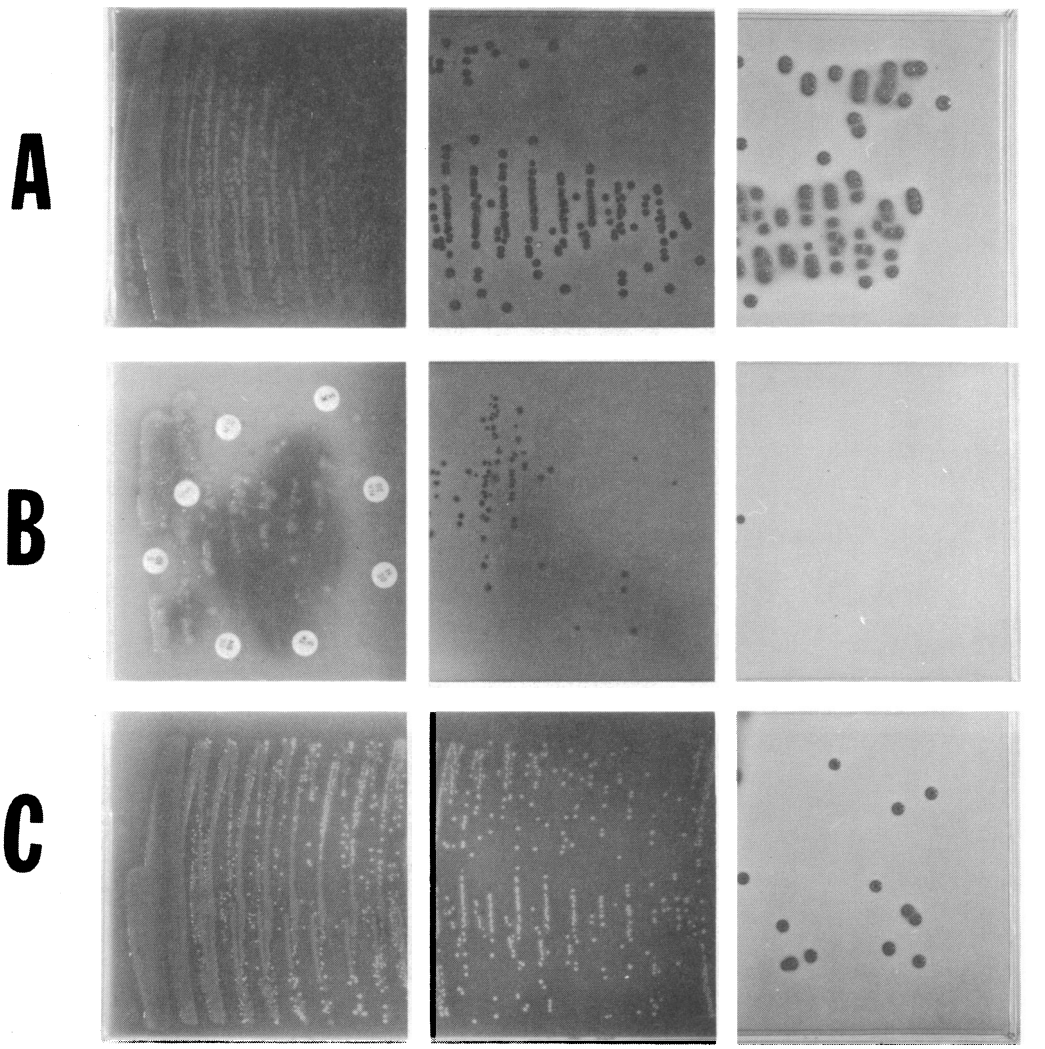

FIG. 6. *Examples of media combinations with an inoculating loop. (A) E. coli on selective and differential media; (B) urinary tract infection (9 × 10⁶ cells/ml) which aided in identification of E. coli and antibiotic susceptibility pattern. Sequence of media in A and B is blood agar, eosin methylene blue agar, and MacConkey agar. (C) Tray containing blood agar, phenylethyl alcohol agar, and MacConkey agar to separate a mixture of E. coli and S. aureus. Inoculum for A and C contained 10⁸ to 10⁹ cells/ml.*

phenylethyl alcohol agar to inhibit gram-negative organisms, and MacConkey agar which permitted growth of the gram-negative organisms while inhibiting the gram-positive bacteria. A survey for *S. aureus* nasal carriers was conducted in which trays containing blood agar, tellurite glycine agar, and Colbeck EY agar were streaked with cotton swabs. Of the 20 persons sampled, the two positive carriers were readily identified by colony characteristics on the selective media. In limited studies of clinical specimens, a loopful of urine from suspected urinary tract infections was streaked on trays containing blood agar, EMB, and MacConkey agar. Good isolation of colonies on the selective media occurred, and presumptive identification of the causative agent was possible. At the same time, antibiotic discs deposited on the blood agar section of the tray allowed early determination of the susceptibility pattern, and the use of a standardized loop (0.01 ml) furnished clues to the relative number of organisms in the sample. Further in-depth studies are being planned to determine the overall utility of this instrument in the clinical laboratory.

To minimize airborne contamination, all inoc-

ulations were conducted inside a plastic box containing ultraviolet lights for sterilization between tests. To determine whether organisms were being disseminated during streaking with either the loop or swab, trays of sterile blood agar or TSA were positioned at the sides and bottom of the instrument during the streaking process. Further studies were conducted in a class 100 clean room with a six-channel particle analyzer (Climet Instrument Co., Sunnyvale, Calif.; model C1-201) and 0.80-μm latex particles to simulate bacterial cells. In no instance could evidence of airborne dissemination during streaking be detected by the two methods employed.

In addition, the plastic box housing the inoculator could be equipped with a controlled temperature, i.e., 35 C, and suitable gas mixtures to enhance recovery of fastidious microorganisms. Although we have demonstrated the application of this machine to bacteria, it should also be suitable for other microorganisms, such as yeast and other fungi, as well as to genetic and other studies where large numbers of isolated colonies are desired.

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